MICROSCOPY.[1]

A NEW METHOD FOR THE MICROSCOPICAL STUDY OF THE BLOOD.[2]—The methods hitherto employed in preparing the blood for microscopical examination have aimed either at the production of *fresh* or of *dry* preparations. Preparations of the first class are not permanent, and those of the second class never exhibit the morphological elements intact. Dr. Biondi has worked out a method which combines the advantages, and is free from the defects, of previous methods. The problem was to find the means of perfect *fixation, preservation, imbedding,* and *mounting*—in other words, a method by which the blood could be treated as a solid tissue. The method is equally useful in the study of other organic fluids, and has been successfully employed in tracing the changes that take place in the maturation of the spermatozoa. It may doubtless be used to advantage in the study of Infusoria, as suggested by Biondi.

The point of chief interest in Biondi's method is the use of *agar* as an imbedding material. Agar is a vegetable gelatine, obtained from *Gracilaria lichenoides* and *Gigartina speciosa,* and has already been successfully employed for some time by Koch in bacteriological investigations. Among the different sorts of agar, the columnar form (Säulen-Agar) is considered the best. A perfectly transparent solution is required, in the preparation of which great care must be taken. This may be accomplished in the following manner: Place two parts of agar in 100 parts of distilled water, leaving it to soften for twenty-four hours at the ordinary room temperature; then heat to boiling on the sand-bath until the agar is all dissolved. The evaporation of the water may be checked by closing the flask with a cork provided with a long glass tube. Add carbonate of sodium to the point of weak alkaline reaction, and boil for an hour in a steam-apparatus. Pour the solution into long, slender test-tubes, and leave from twelve to twenty-four hours at a temperature of 50° to 60°C. The solution separates into two layers, the upper of which is quite clear, and this layer alone can be used for imbedding purposes. But clarification must be carried still farther before it is fit for use. The clear portion of the solution is next to be heated to about 40°, white of egg added, the mixture shaken up several times in the course of ten minutes, boiled for an hour in the steam-apparatus, and then

[1] Edited by C. O. Whitman, Director of the Lake Laboratory, Milwaukee, Wis.

[2] D. Biondi. Neue Methode der mikroskopischen Untersuchung des Blutes. Arch. f. mik. Anat., xxxi., 1, p. 103, Dec., 1887.

filtered. The reaction should then be tested, and, if necessary, carbonate of sodium added until the solution is neutralized. Exact neutralization is necessary, in view of the staining fluid to be employed.

It is important that the mass should be kept sterile up to the moment of using, as otherwise a large number of micro-organisms may develop in it, and render it worthless for the finer uses. It is advisable, therefore, to keep the mass in test-tubes, limiting the quantity placed in each to the probable requirements of a single imbedding operation. For a single preparation of the blood five ccm. of the mass is sufficient. The test-tubes should be cleansed with hydrochloric acid and then washed with distilled water. After receiving the agar solution, the tubes are closed with cotton, and then sterilized in the steam-apparatus for half an hour daily on three successive days.

As the preparation of the agar mass is somewhat complicated, much time and trouble may be saved by turning this work over to some apothecary. König of Berlin (Dorotheenstrasse, 29) furnishes the mass prepared as above described.

The best medium of fixation for the elements of blood is a 2 per cent. solution of osmic acid. If a drop of blood from the frog be examined in this medium under the microscope, it will be seen that both the red and the white corpuscles are perfectly preserved in form and structure. The red corpuscles become a little paler than in the living condition, and are slightly browned. The corpuscles of mammalian blood are isolated and seen to greater advantage than in any other medium of fixation. As it is important that the acid should be perfectly clear and free from all impurities, it is well to filter before using.

Method of Procedure.—1. By the aid of a clean pipette, take a little blood from the heart of a frog, and allow two drops to fall into 5 ccm. of osmic acid (2 per cent.). Shake a little—the sooner the better—in order to separate the elements and scatter them through the whole body of the acid. After standing awhile, the blood corpuscles will be found at the bottom of the tube, the deeper layer being formed mainly of red corpuscles, which sink first by virtue of their greater specific gravity. Exposure, one to twenty-four hours.

2. The process of fixation completed, 4 to 5 drops of the mixture of blood and osmic acid are allowed to fall from a pipette into the melted agar, which is kept fluid at a temperature of 35° to 37°C. By rotating the test-tube, the blood corpuscles are distributed through the agar, and then the whole is poured into a paper box, as in the ordinary paraffine method of imbedding. Within a few minutes the mass stiffens, and may be removed from the box to 85 per cent. alcohol for hardening. In three to six

days the mass is hard enough for sectioning, and may be inclosed in elder pith and cut with the microtome.

If finer sections are required than can be obtained in this way, the agar block may be imbedded in paraffine in the following manner: The block is to be transferred from the 85 per cent. alcohol to bergamot oil (twenty-four hours), then direct to soft paraffine kept at a temperature of 45°C. After one to two hours, the imbedding process may be completed in the usual way. As the agar is saturated with paraffine, very fine sections may be obtained; and these may be freed from paraffine with the usual solvents, and then stained.

3. Sections thus prepared may be safely treated with nearly all staining media. Methyl green, methyl blue, fuchsin, safranin, etc., give the most reliable results. The agar itself is stained only by the most intense anilin dyes (*e.g.*, gentian violet), but in such cases it loses its color quickly in alcohol or in any other decoloring fluid.

4. Sections may be clarified, preparatory to mounting, in balsam or damar, in clove oil, origanum oil, bergamot oil, creosote, etc. Xylol alone should not be used, as it causes the sections to curl.

BOVERI'S METHOD OF PREPARING THE EGGS OF ASCARIS MEGALOCEPHALA.[1]—1. The egg-sacks are plunged for a few seconds into boiling absolute alcohol which contains 1 per cent. glacial acetic acid.[2] The eggs are thus killed instantly, and at the same time the egg-membrane is rendered penetrable to the reagents. The alcohol is allowed to cool gradually, and after a few hours the eggs are transferred to pure alcohol, colored, and examined in glycerine or clove oil. This method shows the achromatic spindles and the chromatic equatorial plates, but not a trace of protoplasmic asters.

2. The following mixture was used cold, with excellent results. A saturated solution of picric acid is diluted with twice its volume of water, and then 1 per cent. glacial acetic acid is added.

The egg-sacks are left at least twenty-four hours in this mixture, then washed in 70 per cent. alcohol, stained in Grenacher's alco-

[1] *Theodor Boveri.* Zellen-Studien. Jenaisch. Zeitschr., xxi., 3 and 4, p. 432, 1887.

[2] Van Gehuchten calls attention to the fact that acid alcohol was used by Prof. Carnoy long before Zacharias published his method. Carnoy employed the following mixtures:

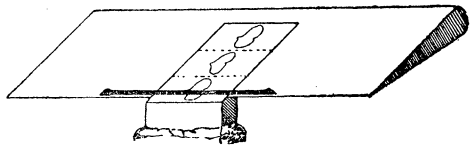
absolute alochol	6 vol.
acetic acid	1 vol.
chloroform	3 vol.

Chloroform renders the action of the reagent more rapid. *Vide*, La Cellule, t. III., f. 1, p. 6 and f. 2, p. 276.

holic borax-carmine (twenty-four hours), transferred to 70 per cent. alcohol plus 1 per cent. hydrochloric acid (twenty-four hours), and finally placed in pure alcohol.

For examination, glycerine is preferred to clove oil. If the egg-sacks are removed from alcohol to a mixture of glycerine (1 part) and absolute alcohol (3 parts), and then allowed to stand until the alcohol has evaporated, the eggs do not shrink. It will be found, however, that the eggs are not all equally well preserved with the cold mixture, owing probably to individual differences in the constitution of the membranes, some being more, others less, permeable to the fixing reagent.

AN INEXPENSIVE SECTION-SMOOTHER.—The cut shows a device for preventing the curling of paraffine sections, which is extremely



simple and easily made. After cutting off the head and point of an ordinary brass pin, fix it parallel to the edge of the knife by pressing its ends into two small pellets of beeswax. The proper elevation is easily determined by testing on the waste paraffine before the object is reached. The pin can only be used with the transverse knife. With the knife set obliquely, a piece of drawn wire will serve the same purpose.—*H. C. Bumpus.*

TABLETS FOR ANATOMICAL PREPARATIONS.—The following information respecting the materials used for mounting tablets in the Museum of Comparative Zoology has been furnished by Professor E. L. Mark:—

For dry objects, various materials have been used at different times: (1) Glass painted on one side; (2) plaster of Paris slabs, white or colored; (3) pasteboard; (4) wood, thin layers glued, with grain running at right angles; (5) slate; (6) cement. The last is worthless. Slate is now preferred.

Samuel Garman was the first to use the plaster tablets for alcoholic preparations. In the Annual Report of the Curator for 1877–8, p. 25, Mr. Garman says: "It is found that by mounting the majority of the Sauria and Batrachia on plaster tablets in jars of alcohol their value for purposes of exhibition is greatly enhanced. This takes considerable labor; but once mounted, they will need no further attention for a long period."

Garman used these tablets in his own room as early as 1875, but they were not introduced into the exhibition rooms until 1877.